Agonist action of taurine on glycine receptors in rat supraoptic magnocellular neurones: possible role in osmoregulation

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1. To evaluate the implication of taurine in the physiology of supraoptic neurones, we (i) investigated the agonist properties of taurine on glycine and $GABA_A$ receptors of supraoptic magnocellular neurones acutely dissociated from adult rats, using whole-cell voltage clamp, (ii) studied the effects of taurine and strychnine *in vivo* by extracellular recordings of supraoptic vasopressin neurones in anaesthetized rats, and (iii) measured the osmolarity-dependent release of endogenous taurine from isolated supraoptic nuclei by HPLC.

2. GABA, glycine and taurine evoked rapidly activating currents that all reversed close to the equilibrium potential for Cl^-, indicating activation of Cl^--selective channels. Glycine-activated currents were reversibly blocked by strychnine (IC_{50} of 35 nM with 100 μM glycine), but were unaffected by the $GABA_A$ antagonist gabazine (1–3 μM). GABA-activated currents were reversibly antagonized by 3 μM gabazine, but not by strychnine (up to 1 μM).

3. Responses to 1 mM taurine were blocked by strychnine but not by gabazine and showed no additivity with glycine-induced currents, indicating selective activation of glycine receptors. Responses to 10 mM taurine were partially antagonized by gabazine, the residual current being blocked by strychnine. Thus, taurine is also a weak agonist of $GABA_A$ receptors.

4. In the presence of gabazine, taurine activated glycine receptors with an EC_{50} of 406 μM. Taurine activated at most 70% of maximal glycine currents, suggesting that it is a partial agonist of glycine receptors.

5. *In vivo*, locally applied strychnine (300 nM) increased and taurine (1 mM) decreased the basal electrical activity of vasopressin neurones in normally hydrated rats. The effect of strychnine was markedly more pronounced in water-loaded rats.

6. Taurine, which is concentrated in supraoptic glial cells, could be released from isolated supraoptic nuclei upon hyposmotic stimulation. Decreases in osmolarity of 15 and 30% specifically enhanced basal release of taurine by 42 and 124%, respectively.

7. We conclude that supraoptic neurones express high amounts of glycine receptors, of which taurine may be regarded as a major natural agonist. We postulate that taurine, which can be released in hyposmotic situations, acts on glycine receptors to exert an inhibitory control on magnocellular neurones during alterations of body fluid homeostasis, implicating an active participation of glial cells in this neuroendocrine regulatory loop.

The neurohypophysial hormones vasopressin and oxytocin are secreted by hypothalamic magnocellular neurones located in the supraoptic and paraventricular nuclei. Both hormones are involved in the regulation of body fluid balance: vasopressin is the principal antidiuretic hormone and oxytocin, although better known for its implication in parturition and lactation, has been suggested to have a natriuretic function (see for review Bourque, Oliet & Richard, 1994). The release of these hormones depends directly on the specific patterns of electrical activity of vasopressin and oxytocin magnocellular neurones (see Bicknell, 1988; Dyball, 1988). Neuronal activity and release of both hormones have been shown to be modulated by peripheral and central osmotic variations (Bourque *et al.* 1994). Central osmosensitive structures involved in this regulation comprise brain areas devoid of the blood–brain barrier, such as the circumventricular organs, that send projections to paraventricular and supraoptic nuclei (Bourque *et al.* 1994). Interestingly, osmotic stimulation of these structures appears to modulate magnocellular neurones firing through selective regulation of excitatory glutamatergic inputs (Richard & Bourque, 1995). However, supraoptic neurones also directly sense the

osmolarity of the environment by means of a particular type of osmoreceptor, stretch-inactivated cationic channels, the activity of which is enhanced or decreased by hyper- or hyposmotic stimuli, respectively (Oliet & Bourque, 1994).

Another factor has long been implicated in the osmoregulation of the brain: taurine, a major amino acid present at high concentrations in the nervous system, is accumulated intracellularly, and is released as an osmolyte by cells facing a hyposmotic environment (see Huxtable, 1992). In the supraoptic nucleus, taurine is predominantly concentrated in glial cells (Decavel & Hatton, 1995), but its role in this structure is unknown. Cultured cortical and cerebellar astrocytes have been shown to release taurine in hyposmotic solution, as well as in response to high $[K^+]$ (Pasantes-Morales & Schousboe, 1988, 1989; Holopainen, Kontro & Oja, 1989; Martin, Madelian, Seligmann & Shain, 1990). However, despite its widespread distribution and its generally accepted role as an osmoregulatory substance, the physiological participation of taurine in neuronal function is still poorly understood. It is believed to be an inhibitory neurotransmitter acting as an agonist on glycine receptors (Betz, 1992). It has also been suggested to interact with at least some subtypes of $GABA_A$ receptors (Horikoshi, Asanuma, Yanagisawa, Anzai & Goto, 1988; Bureau & Olsen, 1991; Quinn & Miller, 1992; Wahl, Elster & Schousboe, 1994). Moreover, the existence of specific taurine receptors has been speculated on the basis of the pharmacological specificity of taurine action on some preparations (Kudo, Akiyoshi & Akagi, 1988), although this has never been clearly demonstrated. Interestingly, applications of high concentrations of glycine and taurine have been reported to weakly hyperpolarize supraoptic neurones in explant preparations, an effect that was antagonized by the convulsant strychnine, suggesting the presence of glycine receptors on magnocellular neurones (Randle & Renaud, 1987). These receptors were not characterized further. Since virtually no glycinergic afferents have been described in this region (Rampon, Luppi, Fort, Peyron & Jouvet, 1996), and all spontaneous and evoked inhibitory synaptic potentials and currents in magnocellular neurones have been attributed to GABAergic transmission (Randle, Bourque & Renaud, 1986; Wuarin & Dudek, 1993), the function of supraoptic glycine receptors remains obscure.

To gain insight into the function of glycine receptors and the role of taurine in the hypothalamo-neurohypophysial system, we characterized the glycine- and GABA-activated currents on dissociated supraoptic magnocellular neurones and studied the action of taurine on glycine and $GABA_A$ receptors. We provide direct evidence for a physiological implication of glycine receptors during changes in water balance *in vivo*, as well as for a hyposmolarity-induced release of taurine from supraoptic nuclei *in vitro*. A preliminary report of these results has appeared in abstract form (Hussy, Deleuze, Pantaloni, Desarménien & Moos, 1996).

METHODS

In vitro electrophysiological recordings

The method used to dissociate supraoptic neurones from adult male Wistar rats (200–300 g; Depré, St Doulchard, France) was derived from that described by Lambert, Dayanithi, Moos & Richard (1994), with modifications. After decapitation without anaesthesia, the brain was removed, and the supraoptic nucleus areas lateral to the optic chiasm were rapidly dissected, cut into three or four pieces, and incubated for 40–45 min in 4 ml oxygenated Locke solution (mM: NaCl, 140; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; KH_2PO_4, 1·2; Hepes-Na, 10; glucose, 10; pH 7·4) containing proteases X and XIV (1 mg ml^{-1} each; Sigma) and deoxyribonuclease I (650 units ml^{-1}; Sigma), at room temperature (22–25 °C). After thorough rinsing, the cells were mechanically isolated by gentle trituration and plated onto 35 mm tissue culture dishes (Costar). These preparations contain supraoptic magnocellular neurones, as well as glial cells and smaller neurones originating from regions adjacent to the supraoptic nucleus. Neurones with a large soma (15 μm or more in diameter) and residual neuritic processes were chosen for recordings. Such large cells have been previously identified as magnocellular neurones by immunostaining against vasopressin and oxytocin (Oliet & Bourque, 1992; Lambert *et al.* 1994).

Recordings were performed within 3 h of dissociation, at room temperature, under voltage clamp in the whole-cell configuration of the patch-clamp technique. Electrodes were pulled from borosilicate glass on a vertical puller (List 3P-A) and had a 4–7 MΩ resistance when filled with the internal solution. Currents were amplified with a List EPC-7 amplifier, filtered at 60 Hz with a Cyberamp signal conditioner (Axon Instruments) and digitized at 133 kHz using pCLAMP software (Axon Instruments). The capacitance of the cells was measured just after the establishment of the whole-cell configuration using a 10 mV step potential from a holding potential of −80 mV, with filtering and acquisition rates of 6 and 30 kHz, respectively. Series resistance was systematically compensated to 70–80%. Intracellular solution was (mM): CsCl, 55; $CsCH_3SO_3$, 75; $CaCl_2$, 0·5; EGTA-Na, 5; Hepes-Na, 10; ATP-Mg, 4; GTP–Tris, 0·5; phosphocreatine, 14; leupeptin, 0·1; pH 7·2; osmolarity, 295 mosmol l^{-1}; and 50 units ml^{-1} creatine phosphokinase was added just before the experiments. This solution helped to prevent or considerably slow down the run-down of $GABA_A$ currents observed in the absence of phosphocreatine and creatine phosphokinase (data not shown). Glycine- and taurine-activated currents did not show appreciable run-down over the recording time. Cells were continuously perfused with the external solution (mM): NaCl, 140; KCl, 3; $MgCl_2$, 1; $CaCl_2$, 2; Hepes-Na, 10; glucose, 10; pH 7·4; osmolarity, 295–305 mosmol l^{-1}. The theoretical equilibrium potential for Cl^- ions was −24·6 mV. Tip potential error was estimated according to Neher (1992) and corrected for (on average −8 mV). When 3 and 10 mM taurine were to be used, an equal amount of glucose was substituted to avoid changes in osmolarity. All drugs and chemicals were purchased from Sigma, except gabazine (SR 95531), purchased from RBI (Natick, MA, USA).

Agonists were applied using a computer-controlled, valve-operated (General Valve, Fairfield, NJ, USA) U tube perfusion system attached to a peristaltic pump (Gilson, Villiers-le-Bel, France). Antagonists were applied in the bath 1 min before the application of a solution containing both the agonist and the antagonist. Data were analysed using Origin software (Microcal Software, Inc., Northampton, MA, USA). Dose–response relationships were fitted with the Hill equations $I = 1/(1 + (EC_{50}/A)^{n_H})$ and

$I = 1/(1 + (A/IC_{50})^{n_H})$ for agonists and antagonists, respectively, where EC_{50} and IC_{50} are the concentrations of half-maximal activation and inhibition, A the drug concentration and n_H the Hill coefficient. Agonist dose–response curves were constructed for individual neurones, and current amplitudes were normalized to the maximal value assessed by the theoretical fit. Normalized values of different cells were then averaged to obtain the mean dose–response curve. Antagonist dose–response curves were constructed by averaging the ratios of currents in the presence and absence of each concentration of antagonist obtained in several different cells. All values are expressed as the means ± S.E.M.

In vivo electrophysiological recordings

Electrical activities of supraoptic vasopressin neurones were recorded extracellularly from lactating (L8–13) or male Wistar rats (250–350 g; Depré) anaesthetized with urethane (ethyl carbamate, 1·2 g kg^{-1} I.P.) according to the method described previously (Moos, 1995). Strychnine and taurine were dissolved in an artificial cerebrospinal fluid solution (mM: NaCl, 126·5; $NaHCO_3$, 27·5; KCl, 2·4; KH_2PO_4, 0·5; $CaCl_2$, 1·1; $MgCl_2$, 0·83; Na_2SO_4, 0·5; glucose, 5·9 g l^{-1}; pH 7·4), and were injected in the vicinity of the recorded neurones using double-barrelled pipettes connected to a pneumatic picopump (WPI Inc., Sarasota, FL, USA). Pressure was adjusted to 40–100 kPa and pulses of 20–40 ms were delivered at 1 Hz during 100–300 s. The outflow of the pressure pipette was checked under binocular observation at the end of each experiment.

In lactating rats, a suckling stimulus was applied to trigger the milk-ejection reflex, which allowed the differentiation of oxytocin from vasopressin neurones (Poulain & Wakerley, 1982). The latter could either be silent or display typical phasic activity (i.e. succession of active periods and silences), but never expressed bursts correlated with suckling-induced milk ejections like those displayed by oxytocin neurones (Poulain & Wakerley, 1982). In male rats, only neurones with clear phasic activity were considered as vasopressin cells. Recordings were performed either in water-loaded rats (after I.P. injection of 20 ml distilled water), or in normally hydrated rats (recordings performed during the first 4 h after anaesthesia). The parameters used to analyse the firing of vasopressin neurones were: F, the mean firing rate during a given period (in spikes s^{-1}); f, the firing rate during active periods (in spikes s^{-1}); and the activity quotient, Q, the proportion of time during which a cell is active (i.e. with interspike intervals shorter than 2 s). These parameters were calculated over periods of 300 s taken before and during drug application. Statistical significance was assessed using Student's paired t test.

In vitro release of endogenous taurine

Supraoptic nuclei were dissected as described above and put in oxygenated Locke solution at 4 °C. Tissues were then carefully freed of residual optic tracts and blood vessels, as well as small areas just surrounding the nuclei, to leave only the thin translucid strip of tissue that originally surrounded the optic chiasm (mean

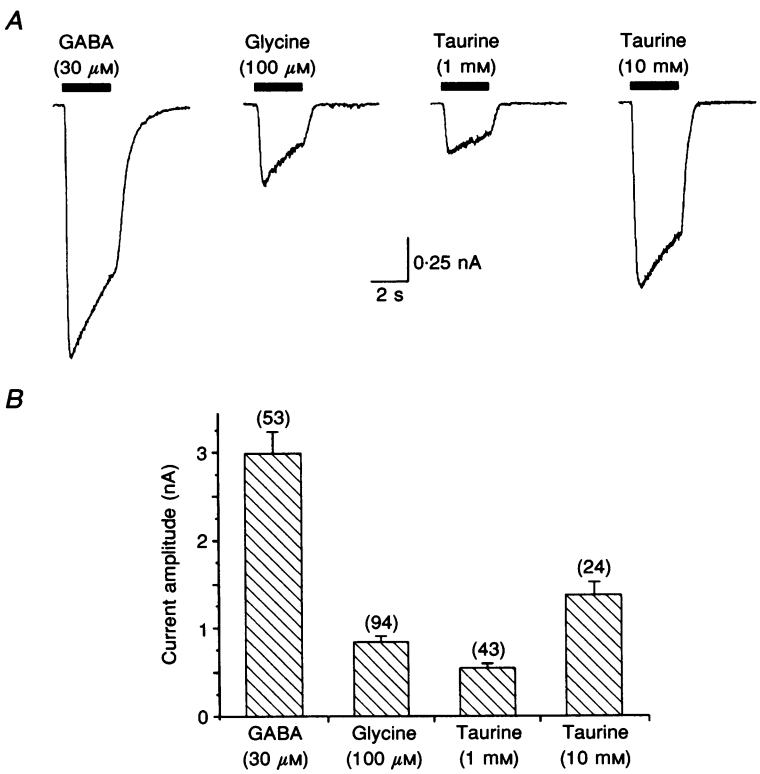

Figure 1. Magnocellular neurones respond to GABA, glycine and taurine

A, currents induced by the three agonists applied at the concentrations indicated. Recordings were obtained from the same cell, at a holding potential of −66 mV. The duration of applications is represented by the bars above the traces in this and all subsequent figures. *B*, averaged amplitudes of the currents activated by GABA, glycine and the two concentrations of taurine. Number of observations is indicated in parentheses.

dimension, 0·4 mm × 0·5 mm × 1·2 mm). They were then incubated in small chambers (250 μl) maintained at 35 °C, and constantly perfused with oxygenated solution at a rate of 140 μl min^{-1}. After 15 min, samples were collected every 2 min (LKB sample collector, Orsay, France) for 14 min and hyposmotic solution (Locke solution with reduced NaCl to obtain the appropriate osmolarity) was applied. As control, a similar reduction of NaCl was compensated for by an equiosmotic amount of mannitol. Two to four samples preceding and following the test application were pooled, lyophilized and processed for HPLC analysis. Samples were submitted to derivatization with phenyl-isothiocyanate, and dissolved in a 60 mM ammonium acetate buffer (pH 6·6), to which was added 4% acetonitrile. HPLC separations were carried out under isocratic conditions with a 5 μm Spherisorb ODS2 C_{18} column, 25 mm × 2·4 mm (PhaseSep, Pessac, France), at room temperature, and at a flow rate of 1 ml min^{-1}. Peaks were detected with a model 163 UV detector (Beckman) at 254 nm. Tissues were collected at the end of each experiment and protein content evaluated.

RESULTS

In vitro characterization of glycine receptors

GABA, glycine and taurine activate Cl^- currents. Voltage-clamp recordings were carried out on 108 supraoptic magnocellular neurones, which had a mean capacitance of 23·7 ± 0·5 pF and a mean input resistance of 1·2 ± 0·1 GΩ. All cells tested responded to the application of GABA (30 μM) by a large inward current at a holding potential of −66 mV (Fig. 1), with an averaged peak amplitude of −2·98 ± 0·25 nA ($n = 53$). The current decreased during the continuous presence of GABA, reflecting receptor desensitization. Application of glycine (100 μM) resulted in a similar current in 97% of the cells (94 out of 97), although generally of smaller amplitude than GABA-activated currents (−0·83 ± 0·07 nA). As assessed from dose–response relationships (see below), the currents activated by 100 μM glycine and 30 μM GABA represented 90 and 65% of the respective maximal currents. Based on these values, we compared the maximal amplitude of the currents in cells onto which both agonists were applied ($n = 44$). Glycine-activated maximal currents averaged 31 ± 5% of those induced by GABA, although this ratio was highly variable from cell to cell, ranging from 1 to 118%. Taurine also activated currents in all glycine-responsive neurones with peak amplitudes of −0·54 ± 0·05 nA at 1 mM ($n = 43$) and −1·38 ± 0·15 nA at 10 mM ($n = 24$; Fig. 1).

The reversal potentials of the currents activated by GABA, glycine and taurine were determined by application of the drugs at different holding potentials to study current–voltage (I–V) relationships. Figure 2 shows the result of such an experiment in a single neurone. The I–V relationships of the currents elicited by the three transmitters were linear in the voltage range examined. The reversal potentials were −26·0 ± 1·7 mV for GABA ($n = 6$), −26·2 ± 1·0 mV for glycine ($n = 5$), and −26·9 ± 1·7 mV for taurine ($n = 4$). These values are close to the theoretical equilibrium potential for Cl^- ions in our experimental conditions (−24·6 mV), and

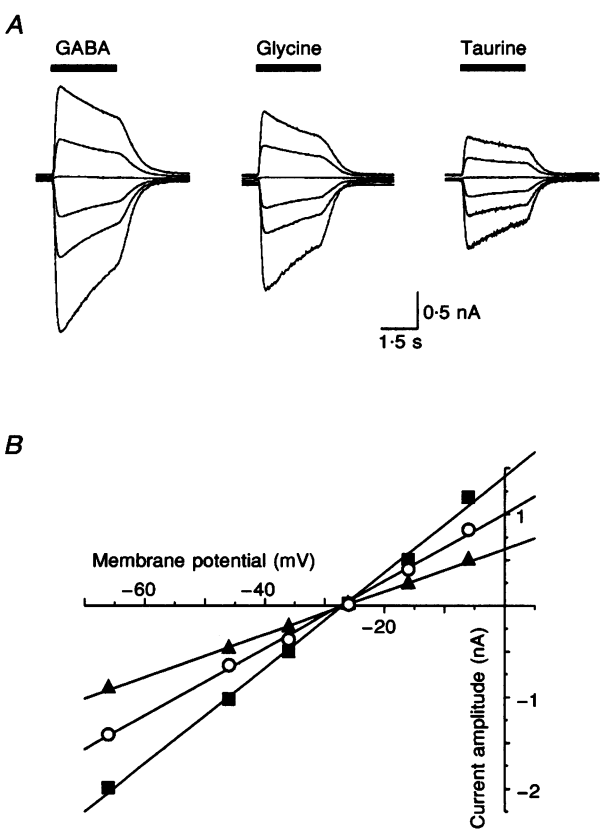

Figure 2. GABA, glycine and taurine activate Cl^- currents

A, responses to GABA (30 μM), glycine (100 μM) and taurine (1 mM) in the same cell, at different holding potentials (bottom to top: −66, −46, −36, −26, −16 and −6 mV). *B*, current–voltage relationships of the currents shown in *A*, activated by GABA (■), glycine (○) and taurine (▲). Data points were fitted with linear regressions that cut the abscissa at −26·9, −27·2 and −26·5 mV for GABA, glycine and taurine, respectively, values very close to the theoretical equilibrium potential for Cl^- in our experimental conditions (−24·6 mV).

far from that of all other permeable ions present in our solutions, indicating the activation of Cl^--selective channels.

Pharmacological distinction of glycine- and GABA-activated currents. Increasing concentrations of glycine activated currents of increasing amplitude (Fig. 3*A*). Above 300 μM, the amplitude of the response tended to decrease, probably due to the faster and more pronounced receptor desensitization that occurs at high agonist concentrations. The averaged dose–response curve indicated an EC_{50} of 32·5 μM and a Hill coefficient of 1·9. Glycine-induced currents were reversibly blocked by the glycine receptor antagonist strychnine, with an IC_{50} of 35 nM (Fig. 3*B*). They were unaffected by the $GABA_A$ receptor-specific antagonist gabazine (Fig. 3*C*), with the amplitude in the presence of the antagonist averaging 99 ± 1 and 100 ± 1% of control for 1 and 3 μM gabazine, respectively ($n = 3$ and 5).

GABA-activated currents were also dose dependent (Fig. 4*A*). The currents used to construct the dose–response curve were recorded at a holding potential of −46 mV to reduce their amplitude, in order to minimize the voltage error due to residual series resistance. The fit of the data points gave an EC_{50} of 18·5 μM and a Hill coefficient of 1·4 (Fig. 4*A*).

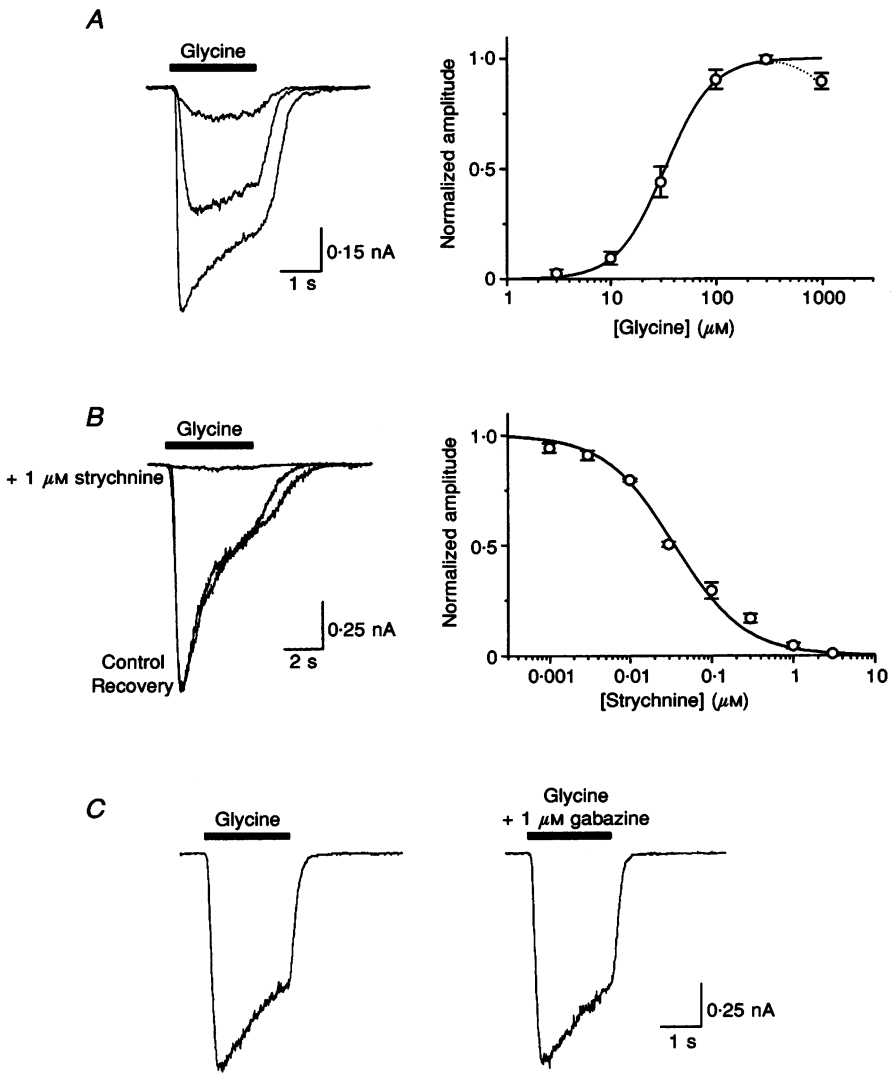

Figure 3. Pharmacological characterization of glycine receptors

A, increasing concentrations of glycine (10, 30 and 300 μM) induced currents of progressively larger amplitude (left). The averaged dose–response curve (see Methods) was fitted with the Hill equation (right) giving an EC_{50} of 32·5 ± 3·4 μM and a Hill coefficient of 1·9 ± 0·2 ($n = 7$ cells). Note the decreased amplitude at 1000 μM glycine, probably due to rapid desensitization of the receptors at high agonist concentration. *B*, the current activated by 100 μM glycine was suppressed by 1 μM strychnine (left). The inhibition was fully reversible after a few minutes wash. The inhibition curve (right) indicated an IC_{50} of 35 ± 2 nM and a Hill coefficient of 1·0 ± 0·1 ($n = 3$–6 cells for each antagonist concentration). *C*, the response to glycine (100 μM) was unaffected by 1 μM gabazine. Holding potential was −66 mV.

GABA-activated currents were reversibly blocked by gabazine (Fig. 4*B*). At 1 μM, gabazine inhibited GABA-induced currents by 86 ± 2% ($n = 3$) and 3 μM blocked them by 94 ± 3% ($n = 3$). Strychnine, at 1 μM, did not affect GABA-induced currents (Fig. 4*C*), having an amplitude of 101 ± 2% of control ($n = 3$). However, 3 μM strychnine slightly reduced $GABA_A$ currents by 19 ± 2% ($n = 4$; Fig. 4*D*). A similar, weak inhibitory effect of strychnine on supraoptic $GABA_A$ receptors has been previously reported (Randle & Renaud, 1987).

Taurine activates primarily glycine receptors. The currents activated by 1 mM taurine were unaffected by gabazine at 1 μM ($n = 3$; Fig. 5*A*) or 3 μM ($n = 4$; data not shown), the current amplitude in the presence of gabazine averaging 99 ± 1% of control ($n = 7$). On the other hand, these currents were suppressed by strychnine, with an inhibition of 95 ± 2% with 1 μM strychnine ($n = 3$; Fig. 5*B*) and of 98 ± 4% with 3 μM ($n = 3$; data not shown). Therefore at this concentration, taurine appeared to activate glycine receptors selectively. This was confirmed by

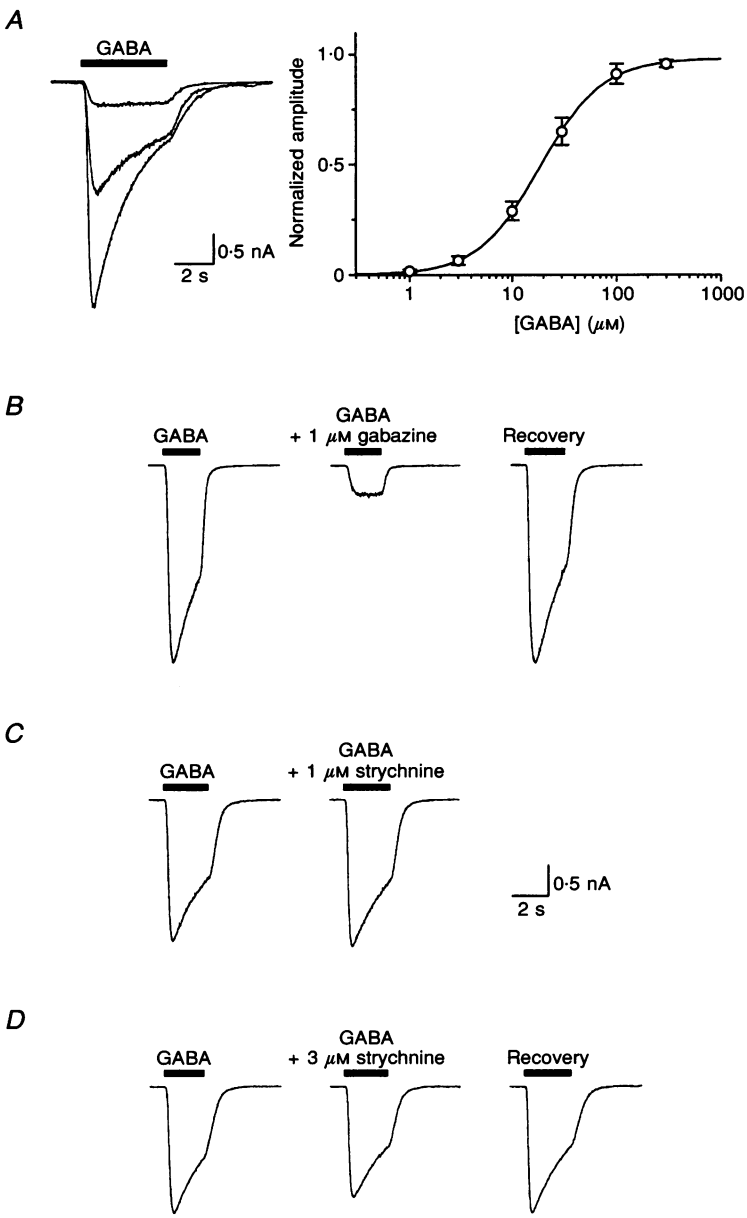

Figure 4. Pharmacological properties of GABA receptors

A, dose dependency of the currents activated by GABA (left). Concentrations were 3, 10 and 30 μM. Holding potential was −46 mV. The averaged dose–response curve for GABA (right) yielded an EC_{50} of 18·5 ± 0·5 μM and a Hill coefficient of 1·4 ± 0·1 ($n = 5$ cells). The response to GABA (30 μM) was reversibly inhibited by 1 μM gabazine (*B*), but was unaffected by 1 μM strychnine (*C*), and only weakly inhibited by 3 μM strychnine (*D*). Holding potential in *B*, *C* and *D* was −66 mV.

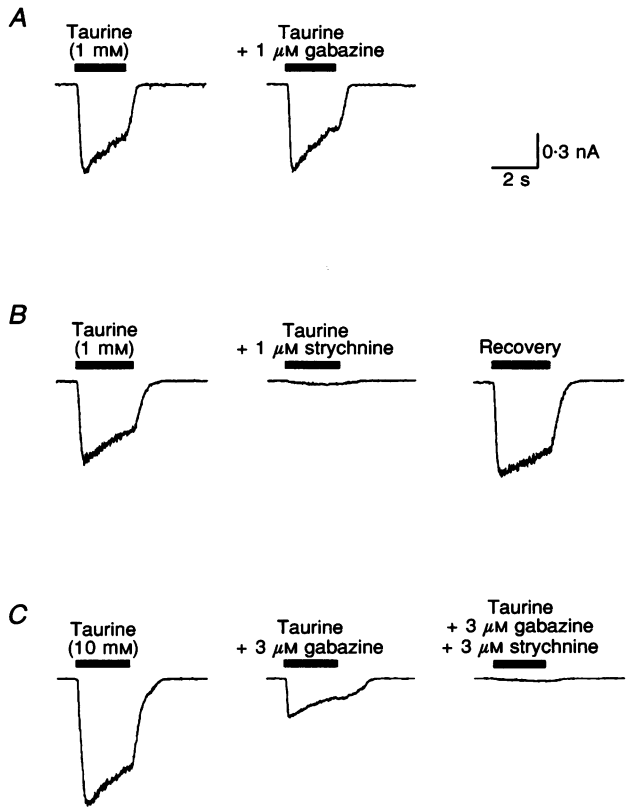

Figure 5. Effects of gabazine and strychnine on taurine-activated currents

The response to taurine (1 mM) was unaffected by 1 μM gabazine (*A*), but was strongly and reversibly inhibited by 1 μM strychnine (*B*). *C* shows that, on the other hand, the response to 10 mM taurine was antagonized by gabazine (3 μM), the residual current being blocked by strychnine (3 μM). Holding potential was −66 mV.

examining the additivity of taurine and glycine currents. When glycine receptors were maximally activated by 300 μM glycine, further addition of 1 mM taurine did not elicit larger currents (Fig. 6*A*), indicating that the two compounds did act through the same receptors. The amplitude of the response to 1 mM taurine together with 300 μM glycine was 94 ± 1 % of that observed with 300 μM glycine alone ($n = 4$). In contrast, the current induced by the co-application of 300 μM glycine and 30 μM GABA was larger than the currents activated by each compound alone, showing the additivity of the two receptor responses (Fig. 6*B*). This confirmed the activation of two distinct populations of channels by glycine and GABA.

The currents induced by 10 mM taurine were often larger than glycine-activated currents (Fig. 1), suggesting that at this concentration, the action of taurine was not limited to the activation of glycine receptors. Indeed, the currents activated by 10 mM taurine were strongly inhibited by 3 μM

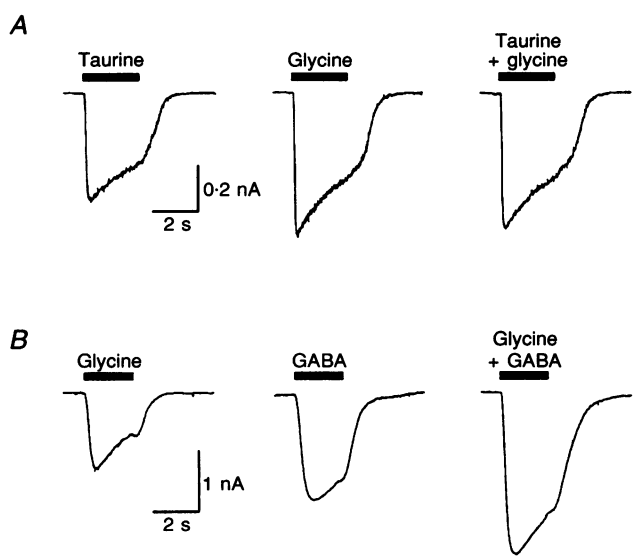

Figure 6. Currents activated by glycine and 1 mM taurine are non-additive

A, the currents induced by the co-application of 1 mM taurine and a maximal dose of glycine (300 μM) were not larger than the currents elicited by each agonist alone, indicating the activation of the same receptor population. *B* shows that, conversely, the amplitude of the currents induced by the co-application of 300 μM glycine and 30 μM GABA was greater than that of the responses to each agonist alone, showing that glycine and GABA act on two different receptors. Holding potential was −66 mV.

gabazine (46 ± 10% inhibition, $n = 13$), the residual current being almost eliminated by further addition of 3 μM strychnine (95 ± 1% inhibition, $n = 3$; Fig. 5C). This indicates that 10 mM taurine activated not only glycine receptors, but also $GABA_A$ receptors on these neurones. The part of the current inhibited by gabazine represented 19 ± 5% of the current elicited by 30 μM GABA ($n = 9$).

We determined the sensitivity of glycine receptors to taurine by constructing the dose–response curve for taurine in the presence of the $GABA_A$ receptor antagonist (Fig. 7). For comparative purposes, the responses to taurine were normalized to that to 100 μM glycine in each neurone. Taurine activated glycine receptors with an EC_{50} of 406 μM and a Hill coefficient of 1·5. The maximal amplitude reached 77% of the peak amplitude of the currents activated by 100 μM glycine, which corresponded to 70% of the maximal glycine receptor activation. This suggests that taurine is a partial agonist of glycine receptors in supraoptic neurones. In agreement with the results described above, the dose–response curve established in the absence of gabazine was similar with taurine concentrations up to 1 mM, but diverged at higher concentrations, at which taurine started to activate $GABA_A$ receptors significantly (Fig. 7B).

In vivo implication of supraoptic glycine receptors in different osmotic states

To evaluate the functional implication of glycine receptors in the regulation of magnocellular neurones, taurine or strychnine was applied on forty-three vasopressin neurones in the supraoptic nucleus recorded *in vivo* in two male and eleven lactating rats. Since similar results were obtained in male and lactating animals, data were pooled. Strychnine and taurine were used at 300 nM and 1 mM, respectively, concentrations shown to affect glycine receptors specifically (see above). Since taurine is involved in osmoregulation, we tested the effects of strychnine on either normally hydrated rats or rats rendered hyposmotic by I.P. injection of 20 ml distilled water. A comparable protocol has been shown to rapidly and sustainedly decrease both plasma and cerebrospinal fluid osmolarity by 15–17 and 12%, respectively (Melton & Nattie, 1983; Melton, Patlak, Pettigrew & Cserr, 1987). Due to the prominent role of vasopressin in the regulation of water balance, we focused our attention on vasopressin neurones.

Effects of strychnine. In normally hydrated rats, vasopressin neurones displayed a variable level of phasic activity. Application of strychnine over 150–300 s in the vicinity of the recorded neurone had a slight, yet significant excitatory effect, the mean firing rate, F, increasing from 2·3 ± 0·4 to 3·8 ± 0·5 spikes s^{-1} ($n = 8$ tests on 6 neurones, $P < 0·001$; Fig. 8A). This activation resulted mainly from an increase in the mean duration of active periods, reflected by an increase in the activity quotient, Q (from 0·39 ± 0·06 to 0·72 ± 0·04, $P < 0·05$). The frequency during active phases, f, was not affected (4·7 ± 0·5 *versus* 5·1 ± 0·5 spikes s^{-1}). In water-loaded rats, vasopressin neurones were markedly inhibited, either displaying a very low level of activity with no obvious phasic pattern, or being quasi-silent. Application of strychnine had a strong, sustained and reversible excitatory effect (Fig. 8B), F increasing from 0·3 ± 0·1 to 2·0 ± 0·2 spikes s^{-1} ($n = 9$ tests on 6 neurones, $P < 0·05$). During activation, neurones often developed phasic activity. The effect of strychnine progressively wore off after the end of the application. These data indicate that a significant basal

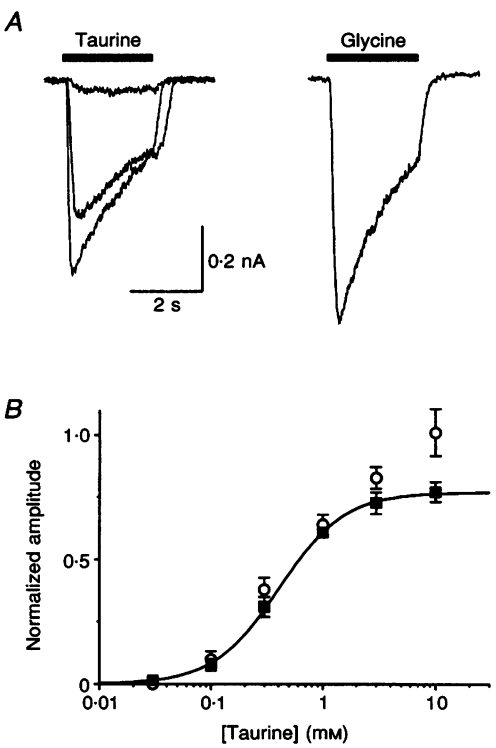

Figure 7. Sensitivity of glycine receptors to taurine

A, currents activated by the application of increasing concentrations of taurine (0·1, 1 and 10 mM) in the continuous presence of 3 μM gabazine (left). The response to 100 μM glycine in the same cell is shown for comparison (right). Holding potential was −66 mV. *B*, dose–response curve for taurine in the presence (■) and absence (○) of 3 μM gabazine. Current amplitudes were normalized to the response to 100 μM glycine. In the presence of the $GABA_A$ receptor antagonist, the fit yielded an EC_{50} of 406 ± 51 μM, a Hill coefficient of 1·5 ± 0·1 and a maximal current of 77 ± 4% of the response to 100 μM glycine ($n = 5$ cells). In the absence of gabazine, an activation of $GABA_A$ receptors by taurine is observed at 3 and 10 mM taurine, as shown by the increased amplitude of the currents at these two concentrations ($n = 7$ cells).

level of activation of glycine receptors is involved in the regulation of the excitability of vasopressin neurones in normal osmotic conditions, and that the decreased activity in response to peripheral hyposmotic stimulus is mediated at least in part through the enhanced activation of these receptors.

Effects of taurine. In rats normally hydrated, taurine was applied onto vasopressin neurones displaying a variable level of phasic activity. As expected taurine had an inhibitory effect in most of the cases (11 out of 13 tests, Fig. 8*C*), decreasing F from $4{\cdot}7 \pm 0{\cdot}5$ to $3{\cdot}0 \pm 0{\cdot}4$ spikes s^{-1} ($P < 0{\cdot}001$). In two other tests, taurine had no significant effect. In neurones showing a clear phasic activity ($n = 5$), taurine decreased the duration of active periods (Q from $0{\cdot}53 \pm 0{\cdot}04$ to $0{\cdot}41 \pm 0{\cdot}03$, $P < 0{\cdot}01$), without affecting f (from $8{\cdot}7 \pm 0{\cdot}9$ to $8{\cdot}8 \pm 1{\cdot}1$ spikes s^{-1}). In the remainder of the neurones, taurine markedly inhibited both Q (from $0{\cdot}88 \pm 0{\cdot}04$ to $0{\cdot}46 \pm 0{\cdot}09$, $P < 0{\cdot}01$) and f (from $5{\cdot}3 \pm 1{\cdot}0$

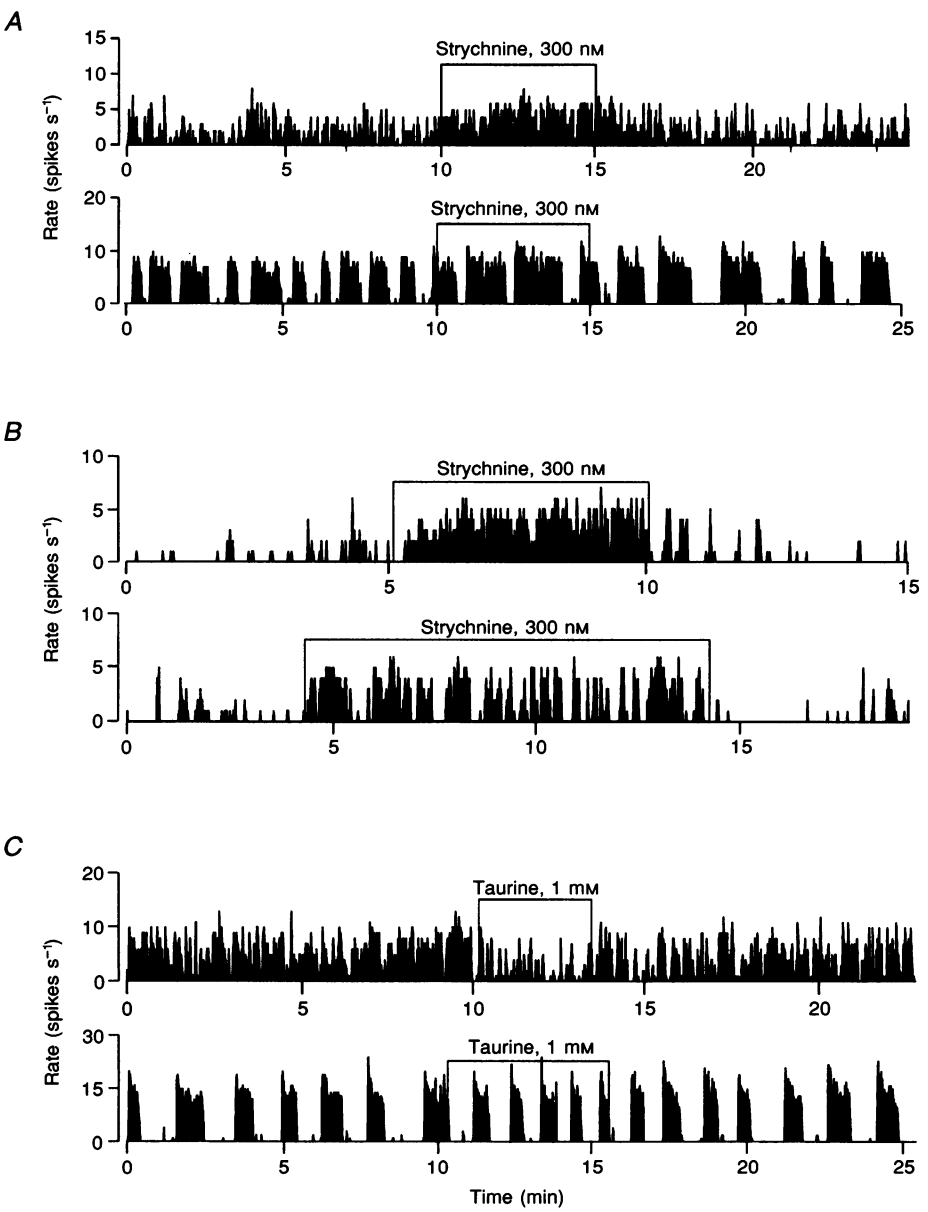

Figure 8. *In vivo* implication of glycine receptors in the control of vasopressin neurone activity
A, firing rate graphs of two vasopressin neurones recorded extracellularly *in vivo* in a normally hydrated rat. Strychnine, applied in the vicinity of the recorded cell for the duration indicated, induced a small increase in firing rate of a non-phasic neurone (top) and increased the duration of active phases in a phasic neurone (bottom). *B*, in a water-loaded rat, vasopressin neurones were quasi-silent. Strychnine elicited a dramatic enhancement of activity, often inducing a phasic pattern (bottom). *C*, taurine, applied in a normally hydrated animal, had the opposite effect to strychnine, decreasing the activity of non-phasic neurones (top) and reducing the duration of active phases in phasically firing neurones (bottom).

to 4·2 ± 0·9 spikes s^{-1}, $P < 0·05$). The effects of taurine were reversible, repeatable and sustained over the application period (up to 8 min).

Endogenous taurine release is specifically enhanced by hyposmotic stimulus

To confirm taurine as a likely candidate for the activation of glycine receptors observed *in vivo*, we assessed the ability of isolated supraoptic nuclei to release taurine in hyposmotic conditions, by measuring the taurine content of the perifusion solution by HPLC. In isosmotic solution (300 mosmol l^{-1}), a small peak of taurine was detectable, which was strongly increased by the application of hyposmotic solution (Fig. 9). The basal release of taurine was 318 ± 123 pmol (mg protein)$^{-1}$ min^{-1} ($n = 11$), which was enhanced by 124 ± 19% by a 30% decrease in osmolarity (212 mosmol l^{-1}, $n = 4$) and by 42 ± 5% by a 15% decrease in osmolarity (252 mosmol l^{-1}, $n = 4$). This effect was specific to the osmotic change, because substitution of 45 mM NaCl by the osmotic equivalent of mannitol (299 mosmol l^{-1}) did not increase the basal release of taurine ($n = 3$, data not shown). The release of the other amino acid agonists of glycine receptors (glycine and β-alanine) was unaffected by the decrease in osmolarity, indicating the specificity of hyposmolarity-induced release of taurine (Fig. 9).

DISCUSSION

Although GABA appears to be the main inhibitory transmitter in the supraoptic nucleus (Randle *et al.* 1986; Decavel & van den Pol, 1990; Wuarin & Dudek, 1993), the presence of strychnine-sensitive glycine receptors has been suggested in supraoptic neuroendocrine cells (Randle & Renaud, 1987). We provide here the first characterization of these receptors in dissociated magnocellular neurones and show their functional *in vivo* implication in the regulation of supraoptic vasopressin neurone activity, especially upon decreases in plasma osmolarity. Moreover, the specific enhancement of taurine release by hyposmotic stimuli in isolated supraoptic nuclei suggests that taurine may be the natural mediator of glycine receptor activation during osmotic regulation.

Properties of glycine receptors of supraoptic neurones

Glycine activated Cl^- currents similar to those induced by GABA, but which could be distinguished by their sensitivity to strychnine and resistance to gabazine. The additivity of maximally activated glycine currents with those elicited by GABA further indicated the involvement of distinct receptors. These responses to glycine were observed in almost all dissociated neurones, implying the presence of the receptors in both vasopressin and oxytocin cells. Glycine receptor maximal currents were on average about 3 times smaller than $GABA_A$ receptor currents. The sensitivities of glycine receptors to glycine and strychnine in supraoptic neurones are comparable to those reported in other central neuronal preparations (Krishtal, Osipchuk & Vrublevsky, 1988; Akaike & Kaneda, 1989; Enz & Bormann, 1995; Kaneda, Farrant & Cull-Candy, 1995). A slope of the glycine dose–response relationship of about 2 appears also to be a general feature of these receptors, in agreement with the three binding sites for glycine proposed by Betz and collaborators (see Betz, 1992).

We showed that taurine can act as an efficient agonist on glycine receptors. The Cl^- currents induced by up to 1 mM taurine could be blocked by 1 μM strychnine (a concentration that affected glycine receptors selectively) and were insensitive to the $GABA_A$ antagonist gabazine. Moreover, the responses to 1 mM taurine and a maximal concentration of glycine were non-additive. These results demonstrate that taurine activated predominantly glycine receptors on these neurones. The dose–response curve generated in the presence of gabazine yielded an EC_{50} of glycine receptors for taurine of about 400 μM and a Hill coefficient of 1·5, values similar to those found in ventromedial hypothalamic and substantia nigra neurones (Tokumoti, Kaneda & Akaike, 1989; Nabekura, Omura & Akaike, 1996). Taurine activated at most 70% of the maximal glycine-induced current, suggesting that taurine acts as a partial agonist on these receptors. The lower slope of the taurine dose–response curve compared with glycine, as well as the partial agonism

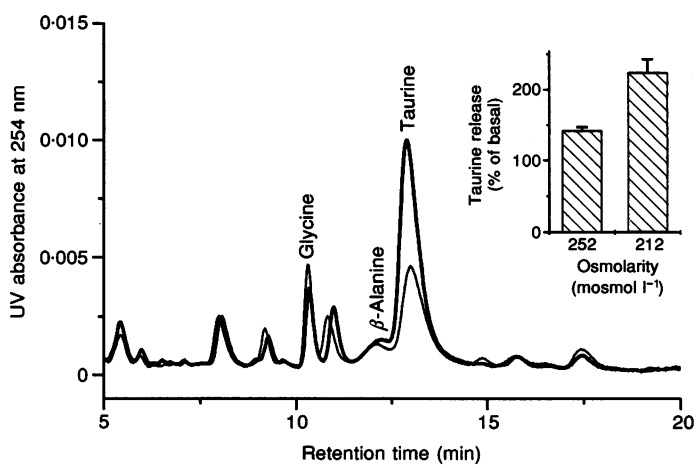

Figure 9. Hyposmolarity-evoked release of supraoptic endogenous taurine *in vitro*

HPLC amino acid analyses of the perfusion medium before (thin line) and 4–8 min after the application of a solution made 30% hypotonic (thick line) by partial removal of NaCl (212 mosmol l^{-1}). Only taurine release is enhanced, glycine and β-alanine being unaltered. Inset, percentage increases in taurine release by 15% (252 mosmol l^{-1}) and 30% hypotonic media (mean of 4 experiments in each condition).

of taurine, are reminiscent of the results obtained with heterologous expression of cloned glycine receptor subunits (Schmieden, Kuhse & Betz, 1992).

Activation of $GABA_A$ receptors by taurine

We showed that high concentrations of taurine (3–10 mM) also elicited an additional current that could be fully blocked by gabazine, indicating that $GABA_A$ receptors have a low sensitivity to taurine. Therefore, taurine can act on both glycine and $GABA_A$ receptors of supraoptic magnocellular neurones, although activation of the former would be the prominent mediator of the effect of taurine in this structure. Such interaction between taurine and both inhibitory transmitter receptors has been previously postulated. Expression of $polyA^+$ mRNA from various brain regions in *Xenopus* oocytes has shown that the $GABA_A$ receptor antagonist bicuculline partially inhibited the currents induced by high concentrations of taurine, GABA- and taurine-activated currents displaying cross-desensitization (Horikoshi *et al.* 1988). In addition, inhibition by taurine of high K^+-evoked release of aspartate in cultured cerebellar granule cells has been shown to be partially sensitive to bicuculline (Wahl *et al.* 1994). Moreover, taurine has been reported to compete for GABA and muscimol binding to $GABA_A$ receptors (Malminen & Kontro, 1986; Bureau & Olsen, 1991) and to stimulate benzodiazepine binding (Bureau & Olsen, 1991; Quinn & Miller, 1992). All these results have been interpreted as taurine acting as a probable partial agonist at $GABA_A$ receptors. Our demonstration of the activation of supraoptic $GABA_A$ receptors by taurine provides a confirmation of this agonist action in a neuronal preparation. Even though high concentrations of taurine were required, this effect may be physiologically relevant. Indeed, intracellular concentrations of taurine of 10^{-2} to 10^{-1} M have been reported (e.g. Kimelberg, Goderie, Higman, Pang & Waniewski, 1990; see Huxtable, 1992) and release of taurine could lead to millimolar concentrations in restricted intercellular areas (see below).

Implication of glycine receptors in the regulation of the activity of vasopressin neurones

We investigated the functional implication of glycine receptors by *in vivo* extracellular recordings of vasopressin neurones both in normally hydrated rats and in rats which were rendered hyposmotic by I.P. injection of distilled water. Local injection of 300 nM strychnine, a concentration that specifically blocks glycine receptors, increased the basal activity of vasopressin neurones, increasing selectively the duration of the active phases of phasically bursting neurones. Application of 1 mM taurine had strictly opposite effects. The effect of strychnine was markedly greater in hyposmotic animals, when vasopressin neurones display a reduced level of activity. This indicates an important role of glycine receptor activation in the regulation of basal activity of vasopressin neurones in normally hydrated rats, as well as a direct implication in the decreased activity of these cells following peripheral hyposmotic stimulation. However, these *in vivo* data do not permit the identification of the natural agonist responsible for receptor activation, nor do they allow the exclusion of the possibility for the involvement of presynaptic receptors. Our *in vitro* results indicate the presence of glycine receptors on all magnocellular neurones, and therefore on both vasopressin and oxytocin neurones; we are presently investigating the implication of these receptors in the regulation of oxytocin cell activity.

If glycine receptor immunoreactivity has been reported in the supraoptic nucleus (van den Pol & Gorcs, 1988), no glycinergic afferents to this area have been described that could account for the large expression of the receptors in all magnocellular neurones (see Rampon *et al.* 1996). Moreover, all inhibitory postsynaptic potentials and currents recorded in supraoptic magnocellular neurones in explant and slice preparations have been shown to be antagonized by $GABA_A$ receptor blockers, and were therefore attributed to GABAergic transmission (Randle *et al.* 1986; Wuarin & Dudek, 1993). Interestingly, a similar situation is encountered in the cerebellum, where both $GABA_A$ and glycine receptors are present on granule cells, but where spontaneous inhibitory postsynaptic currents appear to be exclusively mediated by GABA (Kaneda *et al.* 1995), even though the terminals of the GABAergic Golgi interneurones which synapse onto granule cells often co-localize GABA and glycine (Ottersen, Storm-Mathisen & Somogyi, 1988). These observations raise the possibility that glycine may not be the natural agonist of glycine receptors in this and other higher brain structures. In this regard, activation of supraoptic glycine receptors by taurine is probably of significant importance.

Release of taurine in hypotonic solution

Taurine is present at high concentrations in the brain and can be accumulated in neurones or astrocytes (see for review Huxtable, 1992). In the supraoptic nucleus, taurine has been shown to be prominently concentrated in glial cells (Decavel & Hatton, 1995). In this paper it is shown that the release of endogenous taurine from isolated supraoptic nuclei is strongly increased in hypotonic solution, with significant enhancement by 15% decrease in osmolarity. The physiological relevance of hyposmolarity-induced taurine release is further supported by our (unpublished) observation that a 14 mosmol l^{-1} (5%) decrease in osmolarity significantly ($P < 0.01$) increased the release of preloaded [^{3}H]-taurine from isloated supraoptic nuclei by $11 \pm 2\%$ ($n = 4$). Since the release of glycine and β-alanine, the two other amino acid agonists of glycine receptors, was not affected, taurine appears to be the likely candidate for the activation of the strychnine-sensitive receptors seen *in vivo* in hyposmotic rats. Due to its astrocytic localization in the supraoptic nucleus, the released taurine is probably of glial origin.

Taurine has been shown also to be released from cultured cortical and cerebellar astrocytes in response to swelling following exposure to hyposmotic solution or high extracellular potassium (Pasantes-Morales & Schousboe, 1988, 1989; Holopainen *et al.* 1989; Martin *et al.* 1990; Kimelberg *et al.* 1990; see Huxtable, 1992). Of particular interest is the

response to hyposmotic stimuli, which can occur with minor changes in osmolarity (10–20 mosmol l^{-1}; Martin *et al.* 1990; Pasantes-Morales, Moran & Schousboe, 1990). Taurine is believed to be used by cells as an organic osmolyte to regulate osmotic pressure. As such, taurine, which is concentrated intracellularly, is released by cells facing a hyposmotic extracellular solution to decrease their internal osmolarity (Huxtable, 1992). However, the action of taurine once released has not been clearly defined.

Conclusions

The large currents induced by glycine or taurine suggest a high expression of glycine receptors in supraoptic magnocellular neurones. These receptors appear to be involved in the control of basal activity of vasopressin neurones and, more specifically, in the decreased activity induced by peripheral hyposmotic stimulus, the latter being likely to enhance the release of intranuclear taurine. We postulate that taurine is released by glial cells in hypotonic conditions and acts primarily on glycine receptors to inhibit supraoptic vasopressin neurones, therefore limiting the release of vasopressin in the blood. Thus, not only would taurine serve as an osmoregulator of individual cells, but it would also play an important role in the neuronal circuitry involved in the regulation of the whole body osmolarity. Furthermore, this would imply an active direct involvement of glial cells in a neuroendocrine regulatory loop participating in the maintenance of water balance, a role generally thought to be restricted to neurones. Interestingly, the dynamic implication of hippocampal astrocytes in a glutamate-mediated regulation of synaptic transmission has recently been demonstrated (Winder, Ritch, Gereau & Conn, 1996).

The supraoptic nucleus is an osmosensitive structure involved in the regulation of body fluid balance, and magnocellular neurones respond to hyper- or hyposmotic changes by the opening or closing of stretch-inactivated cationic channels, leading to a depolarization or a hyperpolarization, respectively (Oliet & Bourque, 1994). However, responses to low osmolarity solutions show a limited dynamic range due to closing of all channels by relatively small decreases in osmolarity (Oliet & Bourque, 1994). The release of taurine induced by hyposmotic challenge could lead to a sufficiently high local concentration in the intercellular space to activate glycine receptors (and possibly $GABA_A$ receptors) of adjacent neurones, as suggested by our *in vivo* data. The resulting inhibition of magnocellular neurones could complement and even supply the effect of inactivation of pressure-sensitive channels. Osmosensitivity of supraoptic magnocellular neurones may then be seen as the integration of two complementary systems, an excitatory component formed by neuronal osmoreceptors, activated by hypertonicity, and an inhibitory component resulting from the release of taurine by glial cells, sensitive to hypotonicity. The synergistic nature of these two components should allow a higher sensitivity to small variations in extracellular osmolarity.

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